RESTITUTION OF THE LIVER IN *TRITURUS VIRIDESCENS* FOLLOWING PARTIAL HEPATECTOMY

A THESIS

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CHAPTER I

INTRODUCTION

The liver is the largest gland in the body and provides one means by which experimental work can be done. For a period of years regeneration studies on the liver have been carried on particularly among lower vertebrates. Fishbach ('29) was one of the earlier investigators who succeeded in getting regeneration of the hepatic gland. This aroused the curiosity of many investigators as to the method by which restoration is accomplished.

This investigation was undertaken in order to determine whether there is restoration of the liver in Triturus viridescens after approximately 25% of it was removed.

The results of this investigation indicated that restoration of the liver was accomplished by cell proliferation and compensatory hypertrophy.

CHAPTER II

REVIEW OF LITERATURE

McJunkin and Breuhans ('31) studied the effects of partial removal of the liver and the administration of liver substances on restoration of the hepatic cells in rats. Two-thirds of the liver was removed from 22 rats. Injections of homologous macerated liver were given 9 rats and 13 rats were given their own necrotic livers. The livers were made necrotic by the addition of water to them after which they were allowed to dry. They observed that the rate of regeneration of the hepatic cells was increased in the 9 rats that were given injections of homologous macerated liver and the proliferative activity of the liver was greatly stimulated in the 13 rats that were given their own necrotic tissue. Therefore, McJunkin and Breuhans concluded that the dissolved livers contained a type of cell stimulant.

Lacquet ('32) studied whether certain physiological properties of the restored liver after partial hepatectomy, were essentially different from those of the control organ in rats. When 0.25 cc. of carbon tetrachloride was given to a normal rat, lesions of central necrosis and fatty degeneration appeared in the liver after 24 hrs. and recovery was complete within two weeks. When the same dosage of carbon tetrachloride was given to a rat two to 4 weeks after partial hepatectomy the lesions were less marked and recovery was more rapid. Hence, he concluded that the newly restored liver appeared to be more resistant to the toxic influences of the drug than the control organ.

Milne ('09) studied the histology of liver tissue regeneration in rats from which approximately 75% of the liver was removed. His results

indicated that replacement was by a proliferation of liver cells directly, with no transition in type of cell. The chief proliferative activity of the liver cells was manifested in those situated in the outer half of the liver lobule.

WeinBren ('53) studied the effect of bile obstruction on regeneration of the rat's liver by ligation of the bile duct. Two-thirds of the liver was removed from female rats, after which the bile duct was ligated. Immediately after the operation, WeinBren observed that the liver remnant had hypertrophied. Microscopical examination revealed that this was due to proliferation of liver cells. His results indicated that the rate of regeneration of the hepatic cells was not diminished after bile obstruction by ligation of the bile duct.

Higgins and Anderson ('31) studied restoration of the liver of white rats following partial surgical removal. An average of about 70% of the entire liver substance was removed from 104 white rats. They observed microscopically, as compared with the controls, that restoration of the liver began the first day after the operation and cell proliferation continued in waves of activities which reached a peak on the third day. After the 28th day restoration was complete.

Wilson and Leduc ('50) studied the effect of coramine on mitotic activity and growth in the liver of the mouse. Coramine was administered by adding one per cent of it to the diet. A group of 8 experiments was conducted. They observed microscopically that the inclusion of one per cent of coramine in the diet was followed by an increase in the number of mitotic figures, as compared with the controls. It was suggested that the addition of one per cent of coramine to the diet might be of fundamental importance

in the initiation or stimulation of mitosis in the liver.

Fishback ('29) made a morphological and microscopical study of the liver after partial removal in dogs. From 65 to 70% of the entire liver was removed. Care was taken not to leave pieces of necrotic hepatic tissue behind. Regeneration was completed within 6 to 8 weeks after partial hepatectomy. He found that restoration took place in the remaining lobes which hypertrophied. The hypertrophied lobes attained at least four-eighths of the original volume and weight. Microscopical examination revealed that the hepatic cells were swollen and pale.

Mapp ('34) made a histological study of regeneration in the liver of goldfish after partial hepatectomy. Portions of the liver were removed from 5 goldfish. The progressive stages of regeneration in the hepatic gland were studied over a period of 15 days. He found that the first indication of regeneration was the formation of a reticular network from the connective tissue of the remaining gland. Apparently, the reticulum was laid down first in the restorative process and served as a guide for controlling the direction of migration of the hepatic cells. The cells of the regenerated liver appeared larger and more loosely arranged than those of the control liver. The presence of the larger cells, suggested that restoration of the liver took place by compensatory hypertrophy.

Flores ('51) studied the effects of circulation, temperature and ligation of the bile duct on regeneration of the hepatic cells in toads as compared with the controls. Two groups of experiments were performed. In each group of experiments the temperature was kept at 4°C and 30°C. Approximately 60% of the liver substance was removed from toads. The hepatic portal vein was ligated, immediately after which the bile duct was ligated. Flores' results

indicated that the rate of regeneration of the hepatic cells was markedly reduced following ligation of the hepatic portal vein as compared with the controls. The rate of regeneration of the hepatic cells was reduced after bile duct ligation at 4°C, but at 30°C it was not affected. Thus, Flores concluded that ligation of the hepatic portal vein at lowered temperatures affected regeneration of the hepatic cells and only extremely low temperatures affected the rate of regeneration after bile duct ligation.

Jordan and Beams ('30) made histological studies on the effects of partial hepatectomy in Triturus viridescens. They found that restoration was accomplished, but by compensatory hypertrophy and that there was no evidence of cell proliferation. If more than one-half of the liver was removed, the lobular remnants were not restored.

CHAPTER III

MATERIALS AND METHODS

The animals used for experimental purposes were young adult Triturus viridescens which were obtained from Carolina Biological Supply Company, Elon College, North Carolina. Immediately upon arrival, they were placed in clean battery jars which contained conditioned water. They were fed fresh ground beef and changed to conditioned water every other day.

Twenty-five animals were operated on, each was removed from the battery jar and anesthetized in a finger bowl which contained a one per cent aqueous solution of chloretone.

Each animal was partially hepatectomized by removing approximately 25% of the liver. A lateral incision approximately one-half centimeter was made on the ventral surface, left of the mid-line of the body, just posterior to the forelimb. The liver was lifted out and the required portion was removed. Earle's physiological salt solution was applied to the wound to minimize the loss of blood. The incision was sutured with silk thread. The animals were put singly in a battery jar which contained conditioned water. They were sacrificed at intervals of 12 hrs., 24 hrs., 36 hrs., two, 4, 6, 9, 14, 21, 28, 35 and 42 days. This was done by placing each animal in a finger bowl which contained a 5% aqueous solution of chloretone for approximately 45 to 50 minutes. The liver was then removed and fixed in Bouin's, washed, dehydrated, embedded in paraffin, sectioned at 10 μ and stained with Delafield's hematoxylin and eosin according to Guyer ('37).

CHAPTER IV

EXPERIMENTAL RESULTS

A description of the normal hepatic gland of Triturus viridescens will be presented followed by a histological description of the progressive stages in its restorative processes during a period of 42 days following partial hepatectomy.

The Normal Hepatic Gland

The cells of the normal hepatic gland are large with distinct nuclei. A very few mitotic figures are present and not localized in a specific region of the lobule. The cells at the periphery of the lobule are differentiated and they are arranged in a specific pattern. Binucleated and polynucleated cells were not observed (fig. 1).

The Progressive Stages in Regeneration of the Hepatic Gland

STAGE I. Twelve hours following the operation. A very few mitotic figures were present. Figure 2 shows that there was localization of these figures at the periphery of the lobule. The peripheral cells were very concentrated and undifferentiated. The cells that were located in the center of the lobule appeared normal in respect to the nuclei, however, there was an enlargement of the cytosome (fig. 3), as compared with those of the intact gland. The cells were usually uninucleated, however, some polynucleated cells were found.

STAGE II. Twenty-four hours following the operation. There was an increase in the number of mitotic figures, and they were localized at the periphery of the lobule. The nuclei in the centrally located cells were more distinct

than those of the previous stage. Figure 4 shows that there was an increase in the cell size as compared with the cells after twelve-hours.

STAGE III. Thirty-six hours following the operation. There was an appearance of a few polynucleated cells although the cytoplasmic mass of each cell remained the same. A very few mitotic figures were present at the periphery (fig. 5).

STAGE IV. Two days following the operation. There were approximately more than ten times more figures which represented all stages of mitosis in this series than in the control liver. This represented the highest number of mitotic figures present in all stages. The mitotic figures were more concentrated at the periphery, but some were centrally located (fig. 6).

STAGE V. Four days following the operation. There was a sharp decrease in the number of mitotic figures as compared with the previous stage. The cells at the periphery of the lobule were less concentrated and more differentiated than those of the other stages (fig. 7).

STAGE VI. Six days following the operation. There was a decrease in the number of mitotic figures, accompanied by an increase in the number of differentiated cells which were centrally located (fig. 8).

STAGE VII. Nine days following the operation. The hepatic cells in the center of the lobe had decreased in size (fig. 9). There was a slight increase in the number of mitotic figures as compared with the six day stage and a few binucleated cells were observed.

STAGE VIII. Fourteen days following the operation. There was a sharp increase in the number of mitotic figures at the periphery. The other cells in this same region of the lobule were undifferentiated and very concentrated (fig. 10).

STAGE IX. Twenty-one days following the operation. There was a sharp decrease in the number of mitotic figures. The cells in the center of the lobe resembled those of the control tissue (fig. 11).

STAGE X. Twenty-eight days following the operation. There was a slight increase in the number of mitotic figures as compared with the above stage. The cells at the periphery of the lobule were somewhat scattered, and a few mitotic figures were present among them (fig. 12).

STAGE XI. Thirty-five days following the operation. There was a definite decrease in the number of mitotic figures which were located at the periphery. The cells centrally located were normal with large distinct nuclei. There was no concentration of the cells at the periphery of the lobule and they were more or less differentiated (fig. 13).

STAGE XII. Forty-two days following the operation. There was a decrease in the number of mitotic figures in that only a few more were found than in the control liver (fig. 14). The cells at the periphery of the lobule were differentiated and the cells in the center of the lobe appeared much like those in the control tissue.

CHAPTER V

DISCUSSION

This study was undertaken to determine if restoration of the liver is accomplished by an increase in cell proliferation. A mitotic count was made according to the method of Wilson and Leduc ('47) and a graph was constructed of all progressive stages of regenerating livers. This count revealed that two days after the operation, the occurrence of mitotic figures was more than ten times that of the control and cell proliferation was at a peak. This was followed by a decrease in the number of mitotic figures. Fourteen days following the operation there was a sharp increase in the number of mitotic figures which indicated a second period of rapid proliferation. After this time there was a decline in the number of cells in mitosis.

The mitotic figures were localized at the periphery of the lobule and the cells in this region were concentrated. The cells in the center of the lobule were usually large cells that appeared normal with large distinct nuclei. In some of the progressive stages of regenerating livers binucleated and polynucleated cells were found. This suggests that the cells at the periphery of the lobule undergo cell proliferation and move in toward the center of the lobe as differentiated cells.

Maximow and Bloom ('44) reported that 70% of the liver substance could be removed in lower vertebrates, and the remaining liver would be restored in 4 or 5 weeks. The restoration processes were accomplished by cell proliferation.

Mapp ('34) reported that the restorative processes of the liver in goldfish after partial hepatectomy were accomplished by compensatory hypertrophy,

due to enlargement of the hepatic cells. This was also observed in the early stages of these experiments, but the hepatic cells were undifferentiated at the periphery of the lobule and mitotic figures were localized in that region.

The results as indicated by these experiments are not totally in agreement with those of Jordan and Beams ('30). They reported that restoration of the liver in Triturus viridescens following partial hepatectomy was accomplished, but by compensatory hypertrophy only and there was no evidence of cell proliferation. It was observed in these experiments that in the first stages of the restorative processes there was an enlargement of the cells in the center of the lobule. The cell size decreased as the liver was restored and there was evidence of cell proliferation.

CHAPTER VI

SUMMARY AND CONCLUSIONS

1. Normal and regenerating pieces of liver from Triturus viridescens were prepared for the study of the restorative processes.

2. Two days following the operation cell proliferation was at a peak, followed by a decrease in the number of mitotic figures. Fourteen days later there was another sharp increase in the number of cells in mitosis, which suggested a second growth period.

3. There was an enlargement of cell size during the first regenerative stages. The enlarged cells were located in the center of the lobule.

4. It is suggested that restoration was accomplished by cell proliferation and compensatory hypertrophy.

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PLATE I

(Explanation of figures)*

*All figures are photomicrographs.

(Explanation of figures)

Fig. 1. A section through the normal hepatic gland showing the cells at the periphery of the lobule. X 970.

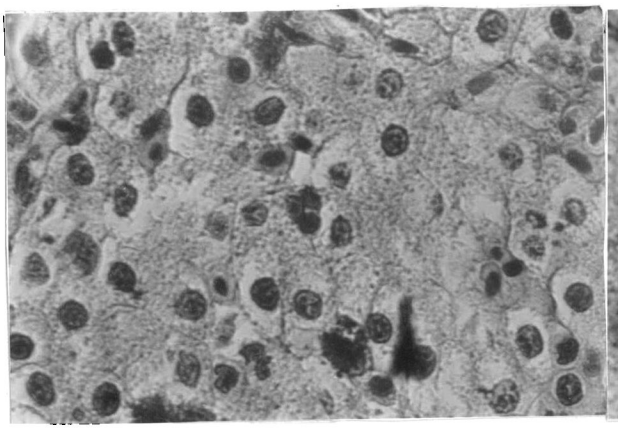
Fig. 2. A section through the liver 12 hrs. following the operation showing the localization of the mitotic figures at the periphery of the lobule. X 970.

Fig. 3. A section through the liver 12 hrs. following the operation showing the concentration of cells at the periphery of the lobule. X 970.

Fig. 4. A section through the liver 24 hrs. later showing the location of the mitotic figure at the periphery. X 970.

Fig. 5. A section through the liver 36 hrs. later showing the localization of the mitotic figure at the periphery. X 970.

Fig. 6. A section through the liver two days following the operation showing the localization of the figure at the periphery of the lobule. X 970.



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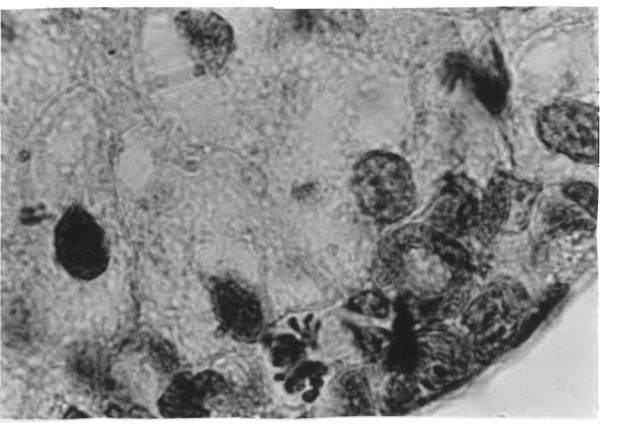

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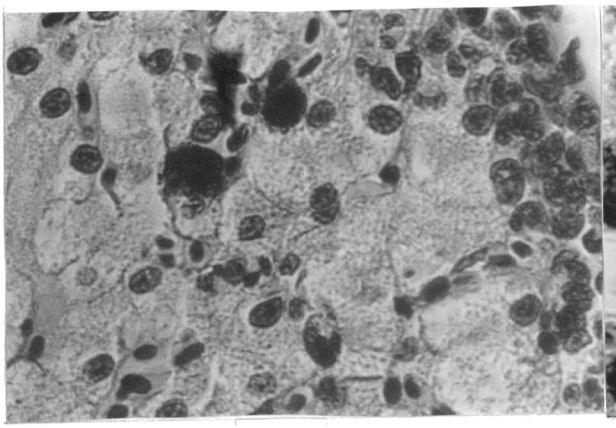

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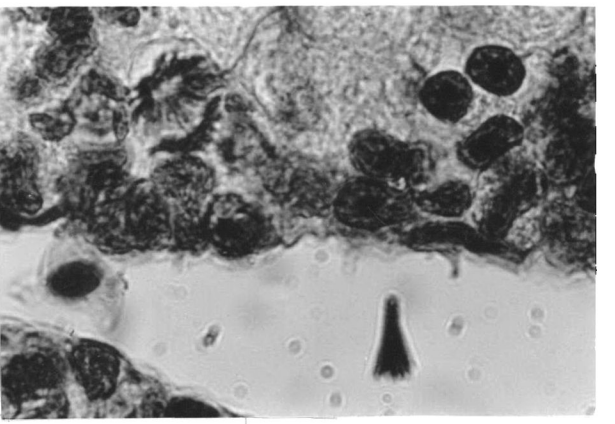

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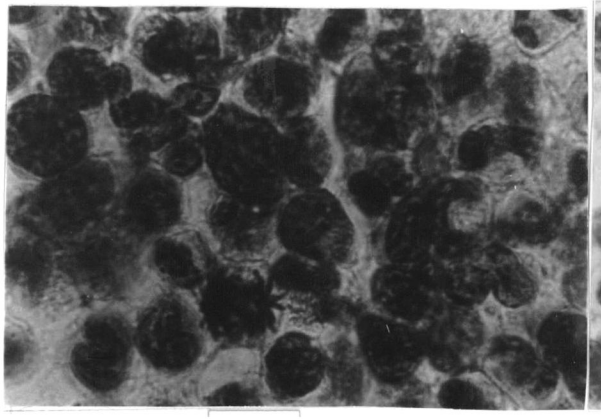

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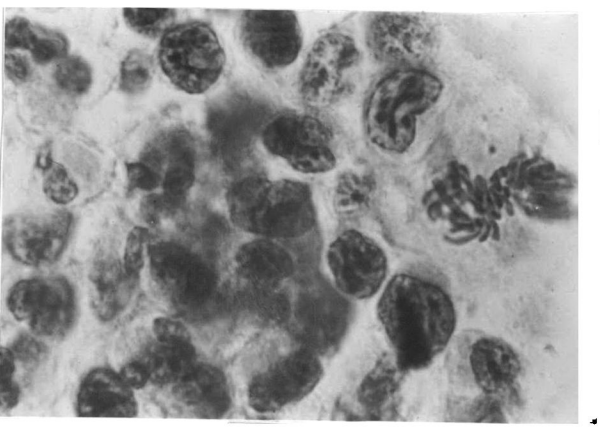

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PLATE II

(Explanation of figures)*

*All figures are photomicrographs.

(Explanation of figures)

Fig. 7. A section through the liver 4 days later showing mitotic figures at the periphery of the lobule. X 970.

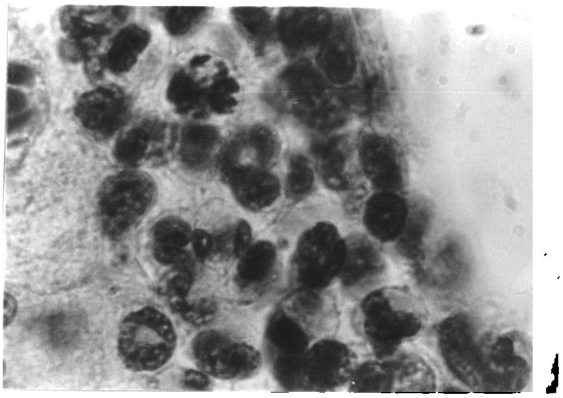
Fig. 8. A section through the liver 6 days following the operation showing the mitotic figure localized at the periphery. X 970.

Fig. 9. A section through the liver 9 days following the operation showing a decrease in size of cells located in the center of the lobule. X 970.

Fig. 10. A section through the liver 14 days later showing the undifferentiated and concentrated cells at the periphery of the lobule. X 970.

Fig. 11. A section through the liver 21 days after the operation showing the cells in the center of the lobule. X 970.

Fig. 12. A section through the liver 28 days following the operation showing the scattered cells and the mitotic figure among them. X 970.



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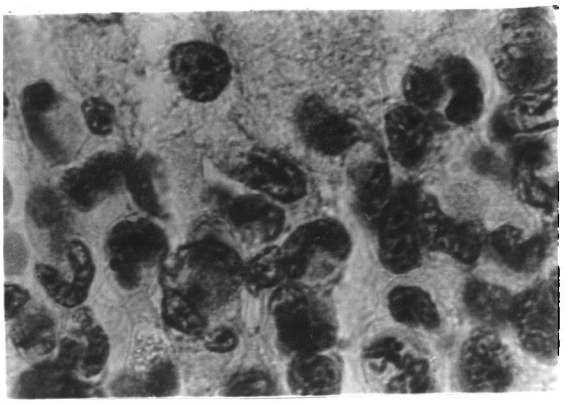

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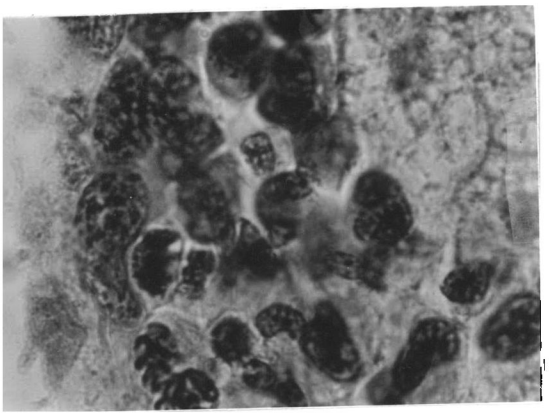

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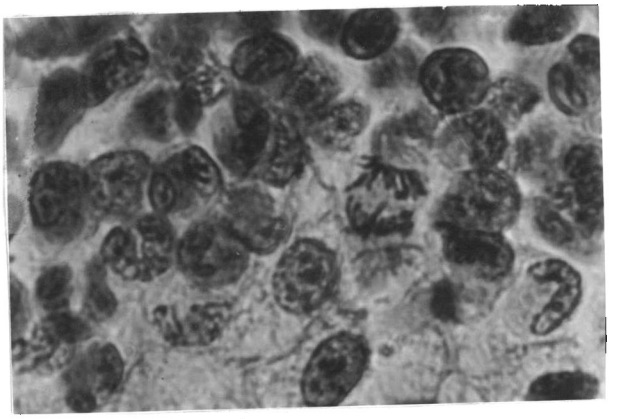

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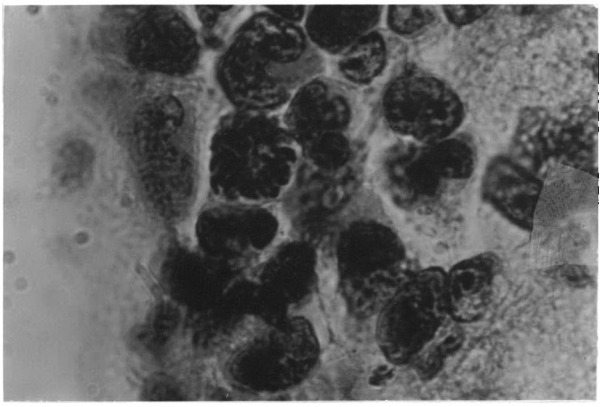

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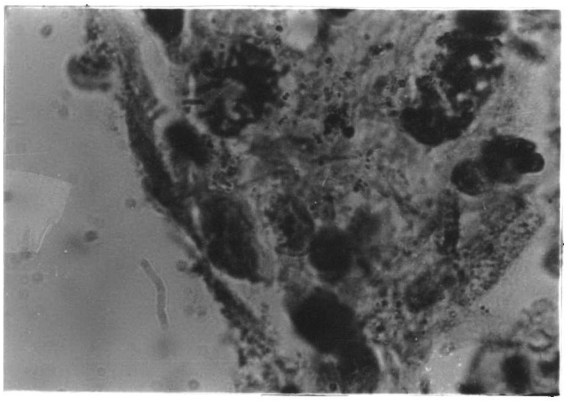

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PLATE III

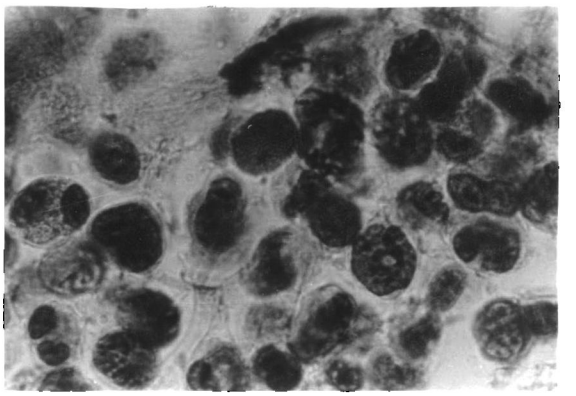
(Explanation of figures)*

*All figures are photomicrographs

(Explanation of figures)

Fig. 13. A section through the liver 35 days following the operation showing the mitotic figure at the periphery of the lobule. X 970.

Fig. 14. A section through the liver 42 days following the operation showing the cells at the periphery of the lobule. X 970.



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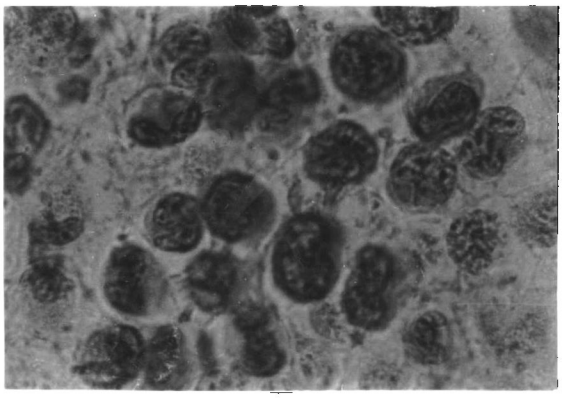

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